DEVELOPMENT OF QUALITY STANDARD AND HEPATOPROTECTIVE EVALUATION OF INDIAN MEDICINAL PLANTS

A synopsis submitted
to in partial fulfilment of requirements
For the degree of
Doctor of Philosophy
In
PHARMACEUTICAL SCIENCES
By

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GANGA NAGAR, MEERUT
JANUARY, 2023

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INTRODUCTION

Hepata is the Greek word for liver. Terms referring to the liver frequently begin with hepato or hepatic. Because the body relies on the liver to regulate, synthesise, store, and secrete many essential proteins, nutrients, and chemicals as well as to purify and clear toxins or unnecessary substances from the body, the liver plays an important role in mechanism, secretion, and storage. **(Arige S et al., 2017).** Liver disease or hepatotoxicity has been characterized as a progressive distortion of hepatocytes and is often characterized as hepatic fibrosis or hepatitis **(iiyas et al., 2016).** the different drug types, detoxifying, and xenobiotics that are produced in the liver. The most severe form of liver disease affects the bile the liver secretes, which has an important function in digestion. Liver failure and acute or chronic hepatitis (anti-inflammatory illnesses) are two possible classifications (degenerative disorder). Infections, autoimmune disorders, excessive alcohol use, and toxic substances like antibiotics, chemotherapeutics, per oxidized oil, aflatoxin, carbon tetra chloride, and chlorinated hydrocarbons can all cause liver illnesses. The liver also contributes in an amazing variety of ways to the body's upkeep, functionality, and homeostatic regulation. It includes all of the biochemical processes involved in development, disease prevention, energy production, reproduction, and the metabolism of nutrients like carbohydrates, proteins, and lipids as well as the excretion of waste metabolites **(Smuckler E. A., 1975).** Drug abuse is one of the main causes of liver diseases, one of the leading factors of morbidity and mortality **(M Nadeem et al., 1997).** Excessive alcohol intake, harmful substances like thioacetamide (TAA), some medications like paracetamol, chemotherapeutic drugs like carbon tetrachloride (CCl4), and serious infections (hepatitis) cause damage to the liver's cells **(S. M Pratap., et al 2014).** Lipid membranes, proteins, and nucleic acids are all damaged by free radicals. hepatic disorders, liver diseases, cancer, inflammatory diseases, and neurodegeneration are examples of pathological conditions that affect the liver **(S. Pal Bhattacharjee, 2014).** The promotion of human health and avoiding the severe morbidity and mortality brought on by liver disease have been strongly stressed in numerous meetings held by national and international disease control agencies and their affiliated organisations. The most effective, necessary, affordable, and readily available natural resources for drug discovery and creation are medicinal plants. The term "traditional systems of medicine" refers to the extensive knowledge of traditional medicine or spiritual therapies, health practises, approaches, manual techniques, and exercise, applied as complementary or alternatives to diagnose, treat, or prevent illnesses, as well as to maintain physical fitness.

Through their catalytic and synergistic potential, traditional medicine and cost effectiveness involving the variety of phytoconstituents play a crucial part in the treatment of diseases.

Increasing utilization of herbal medicine is the alternative treatment in developed countries because of their natural origin, fewer side effects, more affordable, easily accessible. Traditional medicine are Ayurveda, Siddha and Unani system are predominantly used as a plant material. Recently, herbal drugs have gained importance and popularity because of their safety, efficacy and cost effectiveness. The medicinal plants with their other plants habitat also influence their medicinal values but in some cases. It is the most important uses of plant products and their hepatoprotective activity. Liver is a vital organ of vertebrates with multiple functions required in body system. It includes detoxification, biosynthesis, metabolism and many other upto 500 functions **(Zakim, David et al., 2002).** The liver is particularly important for the digestion of drugs and the removal of chemicals from the body, but it is also vulnerable to damage from chemicals. Hepatoprotective activity and chemical agents are both used in various situations to treat liver damage **(Friedman, Scott E et al., 2003).** However, current treatments for hepatotoxicity are not without unfavourable side effects. Traditional medicine uses medicinal plants to safeguard the liver from the hepatotoxicity caused by carbon tetra chloride (CCl4) **(R. Lalfakzuala et al., 2006).** According to predictions made by conventional medicine founded on plant materials, 80% of the world's population will use it **(WHO., 1993).** It alludes to a wide range of herbal medical practises including folk/tribal practises in ayurveda, siddha, unani, and Tibetan medicine that utilised about 1200 plants **(Pushpangadan P., 1995).**

1. DISEASE PROFILE

The liver, which makes up 5% of an infant's body weight and weighs about 1.5 kg in adults, is the second-largest organ in the human body. Right and left lobes as well as quadrate and anterior cingulate accent lobes make up the liver. The right hemisphere is six times bigger than the left. It performs amazing bodily tasks like producing and excreting bile, excreting bilirubin, cholesterol, hormones, and drugs, and metabolising proteins, fats, and carbohydrates. The liver's detoxification (cleansing) process, which removes harmful compounds from our blood, serves the following purposes:

1. Synthesis of protein and bile.
2. Store glycogen, vitamin and iron.
3. Metabolise toxic chemicals and drugs.

(Arige S et al., 2017, Smuckler E. A., 1975, Desai S. N et al., 2012, Jain M et al., 2012, Howida S et al., 2016).

Under the influence of an enzymatic system, biotransformation/drug metabolism is a type of detoxification that is less toxic chemically. The liver's capability performs a number of oxidative metabolisms linked to high cytochrome content in cellular structures. The liver is a particularly vulnerable organ to chemical and drug damage, with symptoms varying from asymptomatic elevation of the liver enzymes to hepatic failure **(M J Coon et al., 1992, T Rajesh et al., 2011)**. Cellular necrosis, a rise in lipid peroxidation, and a decrease in tissue glutathione (GSH) level all contribute to liver damage. Numerous biochemical indicators of serum levels, including triglycerides, cholesterol, bilirubin, alkaline phosphatase, glutamate oxaloacetate transaminase (SGOT/AST), and glutamate pyruvate transaminase (SGPT/ALT) are increased in the liver **(J.S. Mossa et al., 1991, N Mascolo et al., 1998)**. The liver disease commonly observed by the following terms:

(a) Cirrhosis

(b) Necrosis

(c) Both acute and persistent liver failure

(d) A liver disease may cause a decrease in metabolic activity.

Most commonly observed are disorders of bilirubin metabolism and liposis

(i) Fat metabolism-related disorders, such as adipose liver.

(ii) Disorder related to bilirubin such as jaundice.

Different mechanism of action and aetiology of liver can be divided into following parts:

(a) pre-hepatic or hemolytic hepatitis blocked

(b) (Post-hepatic or cholestatic jaundice).

(c) Unconjugated hyperbilirubinemia is accompanied by three conditions: hematogenous, liver jaundice, and cholestasis.

(d) Hereditary jaundice varieties include cholestasis conditions like Gilbert's syndrome, Dubbin Johnson syndrome, Crigler-Najjar syndrome, and Rotor's syndrome, among others.

(e) Chemicals or drug-induced hepatotoxicity are the causes of hepatitis, jaundice, and cancer (Jian W U et al., 1999).

2.1 CLASSIFICATION OF LIVER

Liver can be classified into three categories:

Anti-hepatotoxic drugs: These usually counteract hepatotoxins that cause hepatitis or other liver diseases.

Hepatotropic substances: These are usually supportive of the liver's natural mending process.

Hepatoprotective substances: These serve to avoid liver disease proactively (S. Agarwal., 2001).

2.2 MECHANISM OF ACTION

The mechanisms include an increase in antioxidant levels or a decline in oxidants (ROS formation), inhibition of cytochrome P450s, changes in liver enzyme levels, reduced peroxidation/Lipid peroxidation (MDA), and an increase in glutathione levels or reducing equivalents (B Halliwell et al., 1989).

2.3 TREATMENT OF LIVER DISEASE:

Maintaining fluids and a healthy immune system are essential for treating hepatitis A. In addition to other diseases requiring medical attention to control the illness, gallstones may mandate surgery to remove the gall bladder. Controlling the quantity of protein absorbed from the diet must be essential for avoiding cirrhosis and other diseases. Cirrhosis impairs the liver's ability to metabolism waste products, regulate blood ammonia levels, and prevent hepatic encephalopathy (S Agarwal., 2001).

2.4 Causes of Liver diseases:

- Congenital birth defects and abnormalities of liver present at birth.
- Metabolic disorders and defects in basic body processes.
- Viral or bacterial infections.
- Alcohol or poisoning by toxins
- Some medications are harmful to the liver.

- Dietary problems.
- Pain or damage.

2.5 Symptoms of liver diseases:

- Differences in sleeping patterns.
- Alters in perspective.
- Mild confusion exists.
- Forgetfulness.
- Dizziness of mind
- Changes in personality or attitude.
- Weak judgement and attention to detail.

More severe liver cancer symptoms.

- Shaking of the hands and limbs in an abnormal manner.
- Seizures, agitation, and exhilaration are uncommon.
- Disorientation.
- Strange behaviour and drastic personality changes.
- drowsiness, slurred speech, sluggish movement (Nevah M. I et al., 2016).

2.6 DRUG HEPATOTOXICITY

A wide variety of clinical and pathological liver damage can be brought on by chemical reactions in the liver. Biochemical markers such as alanine transferase, alkaline phosphatase, and bile are used to identify liver damage. These liver disorders include:

(a) The ALT level is more than three times the typical maximum limit (ULN).

(b) The ALP level exceeds what is regarded as normal by more than twice.

(c) A rise in ALT and total bilirubin levels that are over twice what is considered typical **(N. Mumoli et al. (2006); C. Benichou (1990). (N. Mumoli and colleagues, 2006; C. Benichou, 1990).**

2.7 CCL4 induced hepatotoxicity:

Because of the low partial pressure of oxygen in tissues, CCl3 and CHCl2 radicals primarily develop and covalent metabolite binding occurs (Groot De et al., 1998, Masuda and Nakamura., 1990). Steatosis, or fatty liver, is caused by the metabolism of lipids (increased synthesis, reduced transport out of the hepatocyte). High oxygen partial pressure causes lipid peroxidation and CCl4 metabolism to change, pushing cells into death from steatosis **(Kiezka and Kappus., 1980).** Hepatocytes are exposed to CCl4, and toxicity was altered by adding a substance that targets various CCl4 attack stages. Inducers of cytochrome P450, oxygen pressure changes, compounds that cause lipid peroxidation without covalent bonding,

antioxidants, and radical scavengers were some of these substances. Lipoprotein release, covalent binding of activated metabolites to subcellular compartments, and lipid peroxidation's aldehyde production are a few indicators of CCl4 toxicity.

2.8 Acetaminophen

Acetaminophen causes acute liver damage at large doses, resulting in hepatocyte necrosis. At therapeutic doses, the majority of the drug is metabolised to glucuronic, sulphated, and excreted derivatives, and the transitional reactive drug is metabolised, which is eliminated by conjugation with glutathione. However, at excessive doses, the excess is oxidised by the cytochrome P450 at N-acetyl-p-benzoquinone are quickly attach to glutathione, and under excessive conditions of N-acetyl-p **(Ahmad F et al., 2012, Patil B R et al., 2011, Wang A Y et al., 2010. Mc Gill M. R et al., 2012).**

2.9 D-Galactosamine

In terms of morphological and functional features, liver toxin damage is comparable to that caused by viral hepatitis. Hepatocellular necrosis, uracil nucleotide caused by fatty liver, inhibition of RNA synthesis, and protein synthesis can all be brought on by a single dose. The toxicity process can result in decreased ion pump activity and increased cellular membrane permeability, which can release enzymes and raise intercellular Ca2+ concentration **(Simeonova R et al., 2014, Lim H. R. et al., 2000, Raj P.V et al., 2011).**

2.10 LIVER FUNCTION MARKERS

The most crucial indicators for determining liver functionality, generated organ damage, or its cells are its cells. There are no biochemical indicators that can harm the liver, and the combination of several factors that are known to correlate with the liver improves the effectiveness of hepatoprotective models. Markers can be categorised into tests for the viability of hepatocytes, tests for liver excretory function, and tests for synthetic function **(Burkill, Kar D R et al., 2013).**

2.11 COMMON LIVER FUNCTION TESTS:

- It is used to measure particular proteins and enzymes in the blood that are higher or lower than normal levels and suggest a liver issue. Some liver function tests are included.
- **Bilirubin:** The waste substance created during red blood cell breakdown is bilirubin. It is processed by the liver and eliminated through urination. Jaundice, or elevated bilirubin levels, may be a sign of liver injury or specific kinds of anaemia.
- **Alanine aminotransaminase (ALT):** It is an enzyme that is primarily present in the kidney and in higher abundance in the cells of the liver. The body uses it to break down

food and metabolise protein in order to generate energy. Hepatocytes are affected by ALT in harmful ways such as viral hepatitis, ischemic liver injury, and liver damage. Apoptosis and necrosis of liver cells occur as a result of hepatitis C infection, which is caused by ALT, which also raises viral hepatitis types A, B, C, D and E **(Gowda S 2009, Kallei L., 1964, J De., 1999).**

- **Aspartate aminotransferase (AST):** It is an enzyme that is primarily present in the muscles, kidney, and liver or heart in greater concentrations. AST can cause liver injury by releasing blood. Chronic liver diseases and myocardial infarction caused by AST have caused serious tissue necrosis and liver tissue degeneration. When the liver is damaged, certain enzymes that it contains increase in the blood. It analyses the AST/ALT ratio, which is helpful in determining the causes of different types of liver damage **(Gowda S., 2009, H. G Limdi., 2003).**
- **Albumin and total protein:** The liver produces the proteins known as albumin. Total protein and albumin levels that are below normal are signs of liver illness or liver damage. Albumin levels in blood should range from 3.4 to 5.4 g/dL.
- **Gamma-glutamyl transferase (GGT):** A high amount of this enzyme can harm the bile duct or the liver
- **L- lactate dehydrogenase (LD):** Although it can be elevated in many other disorders, the level of the LD enzyme may induce liver damage.
- **Prothrombin time (PT):** It estimates how long it takes for the blood to coagulate. If it persists for a long period, the liver may produce clotting factors, which is a sign of liver failure. Additionally, consuming certain blood thinners like warfarin can cause an increase **(Mayo Medi. Lab., 2019).**

2. LITERATURE REVIEW

Anwar et al., (2023) evaluate antioxidant and hepatoprotective effect of calamus rotang L. ethyl acetate leaf extract at a dose of 350 mg/kg in CCl4 induced hepatotoxic rat and its molecular mechanisms. It measures the pro-inflammatory cytokines, inflammatory regulators and antiapoptotic protein is compared with positive and negative control groups by using immunohistochemical examination. HPLC profiling of the polyphenol content and molecular docking identified compound against antiapoptotic property.

Pandey A et al., (2023) investigated hepatoprotective effect of methanolic extract of Sida veronica folia at a dose of 250 and 500 mg/kg were estimated by using liver function test and serum profile. It not having strong effect on hepatoprotective by lowering the level of serum transaminase (SGPT and SGOT), alkaline phosphate, total bilirubin in the blood and also increase the level of total protein.

Minnandy et al., (2022) studied hepatotoxicity caused by different types of toxic chemicals such as antibiotic and chemotherapeutic agent, paracetamol, thioacetamide, carbon tetra chloride, silymarin, ethanol and excess alcohol intake and microbes. Synthetic drug is treated to liver sickness and cause damage the liver. Herbal remedies are focussed in pharmaceutical industry evolve a safe route for liver disorder and it is very cost, no side effect is compared with synthetic drugs. Therefore, plants are Avicennia alba, Anisochilus carnosus, Baliospermum montanum, Centella asiatica, Clitoria ternatea, Eclipta alba, Justicia adhatada, Phyllanthus emblica, Pisonia grandis and Syzgium cumini are used in hepatoprotective activity.

Chauhan Lavika et al., (2022) studied liver help in metabolise substances having different biochemical pathways include reduction, oxidation, condensation, hydration, hydrolysis and isomerization. Disorder of referenced cycle prompt liver cell injury and have numerous sicknesses. Hepatotoxicity drug, synthetic compound, dietary aggravation or spice-initiated liver harm through hepatotoxins. Various herbo-mineral arrangement in ayurveda and Indian medicine have been examined for hepatoprotective potential to treat liver problems.

Gupta R et al. (2022) invested liver is an important organ that is engaged in metabolism and regulation. Liver failure is a common side effect of many drugs, toxins, and plant-based products and could endanger human life. Hepatic karyopyknotic, eosinophilic, or acidophilic cell plasma, liver injury, and central vein necrosis due to oxidative lipid degradation are all signs of liver failure. The pathogenesis of liver failure is linked to various processing

information, including the activation of Kupffer cells, NK cells, inflammatory mediators, and ROS.

Hota R. N et al., (2022) evaluate hepatoprotective activity of ethanolic extract of L. rugosa aerial part of paracetamol and carbon tetra chloride (CCl4) hepatotoxicity in albino wistar rats. HPTLC analysis are the assessment of phenolic and flavonoid compound and chromatography-mass spectrophotometry (GC-MS). Lipid peroxidation, superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) are determined in liver tissue homogenate. Serum biochemical and histopathological examination and molecular docking analysis were performed to understand molecular mechanism of hepatoprotective activity.

V. Sumitha et al., (2022) studied hepatoprotective activity of methanolic leaf extract of Hyptis capitata H2O2 induced oxidative stress of HepG2 cell lines. The total antioxidant capacity and superoxide radical scavenging activity of the extract were also performed. The extract considerable free radical scavenging activity ($P<0.01$). The extract displayed concentration dependent cell viability with maximum protection (63.97±1.23%) H2O2 induced HepG2 cell lines at 100 µgml-1Hyptis capitata is a herb with significant antioxidant and hepatoprotective potential.

Hussain S et al., (2022) studied the protective effect of methanolic extract of Chenopodium album against carbon tetra chloride induced hepatotoxicity in rats. Chenopodium album is locally used for multiple medical purposes. Methanolic extract of Chenopodium album (whole part) was prepared with Soxhlet apparatus and rotatory evaporator. Antioxidant activity of Chenopodium album were determined by DPPH free radical scavenging assay.

Aramjoo H et al., (2022) investigated the defence mechanism of Sophora pachycarpa seed extract against toxicity caused on by carbon tetra chloride on bodily organ, blood, and biochemical variables. Creatinine, urea, blood nitrogenous nitrogen (BUN), uric acid, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are serum renal and hepatic markers that may be influenced by carbon tetra chloride. MDA levels may also be increased, while CAT, FRAP, GSH, and SOD are reduced in lever and kidney tissues. Before administering carbon tetra chloride, Sophora pachycarpa seed extract significantly reduced these changes, including haematological and histopathological alteration.

Ugwu and Suru et al., (2021) reported Hepatotoxic agents are well characterised by reported carbon tetra chloride (CCl4). Once liver diseases were discovered, it was crucial to evaluate and create hepatoprotective agents for plant sources. A systematic literature review on plants with potential for liver protection that was released between 1998 and 2020 revealed 92 medicinal plants as being particularly effective at preventing the liver damage caused by carbon

tetra chloride in animal models. Herbal plants defend the liver from CCl4 by inhibiting the enzymes that mark the liver and activating the antioxidant capacity of the liver cells while repairing the liver's structural integrity. Traditional and pharmacological uses of plants were supplied by liver. Flavonoids and polyphenol molecules belong to several phytoconstituents that are recommended for liver protection.

F. Padberg et al., (2021) investigated the different cellular populations that make up the liver. Through an indirect co-culture system made up of immortalized primary human hepatocytes and macrophage-derived monocytes, different cell populations can interact (MDMs). IL-8 and TNF-neutralizing antibodies are correlated using time-dependent cytokine secretion of co-culture and single culture, as well as linkage networks of G-CSF, CCl3, MCP-1, CCl20, FGF, TGF-1, GM-CSF, IL-8, IL-6, IL-1, and IL-18. It reveals that IL-8 is involved in the encounter between hepatocytes and macrophages. Transcriptome analysis shows that variations in the ratio of macrophages to hepatocytes may induce its release of supplementary cytokines and chemokines during in the acute phase response, and that the use of diclofenac and LPS increases the amount of MDMs.

Bhatt N et al., (2021) investigated liver disease is prolonged malfunctioning linked to various cellular and biological changes. Indigenous medical systems like ayurveda, siddha, and unani are frequently used to treat liver illnesses. Results of thorough investigation and analysis of therapeutic efficacy and hepatoprotective features. The primary function of ayurvedic, siddha, and unani products and ingredients is the treatment of chronic or serious liver ailments. These systems and scientific data on products and plants show a lot of promise for translating to the growth of new pathways and the creation of new, secure, and efficient therapeutic modalities to treat liver disorders.

Amjad Hussain et al., (2021) studied many severe liver conditions, including cirrhosis, liver disease, liver failure, and hepatitis, are brought on by viruses and hepatotoxic substances. Many novel drugs and their active ingredients are based on natural products. Herbal remedies and medicinal herbs are used to treat a variety of hepatic diseases. But in the worst instances, a single medicinal plant is inadequate. Alkaloids, glycosides, saponin, flavonoids, terpenes, and other medicinal and powerful substances found in these plants have antioxidant and hepatoprotective properties. Silibum marianum or milk thistle (Silymarin), Glycyyhiza glabra (Glycyrrhizin), Picrorhiza kurroa (Picroside I and II), Phyllanthus maderaspatensis (β-sitosterol), Andrographis paniculate (Andrographolide), Trichopus zeylanicus (Polyphenol), Eclipta alba (Ecliptal) and Phyllanthus amarus (Phyllanthin) are sufficient active against certain liver diseases.

Divya Jain et al., (2020) investigated phototherapeutic approach for the development of modern drug can provide various important drug for medicinal plants. Exploration of pure Phytocompound is very expensive and time taken agents. Various plants and their herbal formulation are found to be useful for cure of liver diseases. Number of valuable plants and their formulations show therapeutic efficacy against some chemically induced liver damage, cancer cell lines and animal models. Medicinal plants and their common dietary sources provide protection against liver damage caused by chemicals and oxidative stress mechanism.

Shakya Arvind et al., (2020) studied liver is a crucial organ for the body's energy generation and nutrient metabolism. Additionally, it is essential for the kidneys to process and eliminate dangerous substances and extraneous drugs. Different hepatic diseases like jaundice, necrosis, hepatitis, fibrosis, and cirrhosis are induced by hepatotoxic agents. Ayurveda is a traditional medicine that has been used to cure human illnesses ever since the beginning of civilization. A critical feature of such therapeutic compound used to create successful medications for human diseases like liver disease comes from medicinal plants. As a result, the pharmaceutical industry uses therapeutic plants regularly to create safe medications for the therapy of emerging human diseases.

Reema Srivastava et al., (2018) investigated liver is the body's main organs for metabolism and excretion. The different biochemical pathways used by the human liver to breakdown substances include hydration, condensation, hydrolysis, conjugation, and isomerization. Hepatotoxic disorders can lead to a variety of illnesses, many of which have greater mortality rates globally. Hepatotoxins, which cause liver injury, can be generated medicines, chemicals, diet modification, or herbs. In ayurveda and traditional Indian medicine, there are many herbals and Herbo mineral preparations that have been used for their hepatoprotective properties in treating various types of liver disorders.

Sai Sruthi Arige et al., (2017) studied the most important structures for metabolism and excretion in the human body is the liver. It plays a crucial part in the liver's upkeep, functionality, and homeostasis regulation. All the biochemical processes for nutrient supply, disease prevention, growth, energy generation, and reproduction are involved. The liver's primary roles include the metabolism of carbs, proteins, fats, and calories as well as detoxification, bile secretion, and vitamin storage.

Sathidpak Nantasanti et al., (2015) reported new opportunities for gene and stem cell therapy for treating liver diseases have been facilitated by the development of three-dimensional liver stem cell cultures (hepatic organoids). The dog is thought to be the best model for human liver diseases and allows for long-term canine hepatic organoid culture, which is the undifferentiated

expansion of progenitor cells or differentiated towards properly functioning hepatocytes. Hepatoprotective activity in a large number of animal models is necessary for test efficacy and safety because it decided to share pathological and pathway diseases.

Gunturu Abhilash et al., (2014) studied for rural people, particularly those from developing countries with few healthcare facilities, medicinal plants are essential. It is suggested that 70,000 different plant species, varying from lichens to tall trees, have been used medicinally. This emphasizes the important role of plants for hepatoprotective action. It is helpful for medical professionals, academics, and researchers working in the fields of therapeutic development, pharmacology, and evidence-based alternative medicine to treat various liver illnesses in humans or animals.

Ali E Al-Snafi et al., (2013) studied hepatoprotective effect of medicinal plants against induced oxidative hepatotoxicity, aflatoxin oxidative stress, carbon tetra chloride, D galactosamine, lipopolysaccharide, N-dimethylformamide, oxytetracycline thioacetamide, nitrosodiethylamine, paracetamol, and drug induced hepatic cancer.

Amol P. Pachpute et al., (2013) evaluated the ethanol extract fruit product of the Piper cubeba plant's 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity in eliminating free radicals, reducing power, hydroxyl activity in eliminating radicals, nitric oxide antioxidant activity, and hydrogen peroxide radical scavenging. In a rat model of liver injury caused by carbon tetra chloride, the extract's hepatoprotective qualities are assessed (CCl4). After carbon tetra chloride CCl4 elimination, the ethanolic extract of Piper cubeba significantly altered the serum marker enzymes and total protein. The histopathology of the liver section from the extract-treated animal shows the presence of healthy hepatic cords and the absence of necrosis and fatty infiltration, which together further suggest hepatoprotective action.

Saumendu Deb Roy et al., (2012) investigated Hepatoprotective drugs can be found in significant amounts in medicinal plants. There are both mono- and poly-herbal preparations used for different liver diseases. Over 100 plants are used in therapeutic settings, and there are over 700 mono- and poly-herbal preparations available in the forms of decoction, tincture, tablets, and capsules. Both hepatoprotective medicines and their toxic counterparts have positive effects on the liver. Controlled trials have shown that herbs are truly useful for managing liver diseases and that phytopharmacological study on herbs has been beneficial in treating gall bladder and liver disorders.

Feroz Ahmad et al., (2012) studied Hepatoprotective drugs have been evaluated in both an in vitro and an in vivo liver animal. These methods assess a drug's capacity to protect against and treat liver toxicity (caused by liver hepatotoxin) in experimental rats. In vivo models demonstrate toxic doses or repeated doses of known hepatotoxins are administered harm to livers and experimental animals, but in vitro models of fresh hepatotoxins and effect of standard drug are the same evaluated. Different chemical substances that are commonly used to cause liver damage in animals are also evaluated for their effects on the liver. These substances include alcohol, carbon tetra chloride, paracetamol, acrylamide, Adriamycin, and antitubercular medicines, among others.

Z A Zakaria et al., (2011) studied Rats whom the livers have been injured by paracetamol will be used to evaluate the hepatoprotective impact of virgin coconut oils produced by MARDI. Paracetamol raises liver weight by 3 gm/ka per 100 g of body weight in response to liver injury, demonstrating liver damage. The prevalence of inflammation and necrosis on particular liver sections is revealed by histological observation, which again supports the existence of liver damage.

E A Adewusi et al., (2010) studied In terms of modern medications, there is no effective medication that can both enhance liver functions and shield the liver from damage or aid in the regeneration of hepatic cells. Effective drug alternative and supplementary applications are urgently required. Undoubtedly, the plant kingdom is a useful source of new pharmaceuticals. the plant extract and naturally occurring, chemically defined molecules with hepatoprotective action. Additionally, it contains 58 isolated compounds from higher plants that have been categorized into the proper chemical groups. It displays 107 plants along with information about their families, spread across the globe, plant parts used in assays, and causative agents of liver damage.

3. RATIONALE OF THE STUDY

The most common diseases, hepatotoxicity or liver problems and its consequences, is marked by the progressive distortion of hepatocytes, loss of normal liver function, biochemical imbalance, etc.

Numerous reports of drug-induced liver damage have already been made, and this data has been used to establish which drugs are most likely to safeguard the liver from damage.

The best model for inducing hepatotoxicity and evaluating pharmaceutical active compounds and drugs that have historically been used in the shape of medicinal plants is carbon tetra chloride (CCl4).

Medicinal plants are regarded as a good source for treating many acute and chronic illnesses because of the fact that they are readily available, inexpensive, and have few side effects.

Qualitative and quantitative investigation using HPTLC, LC-MS/GC-MS

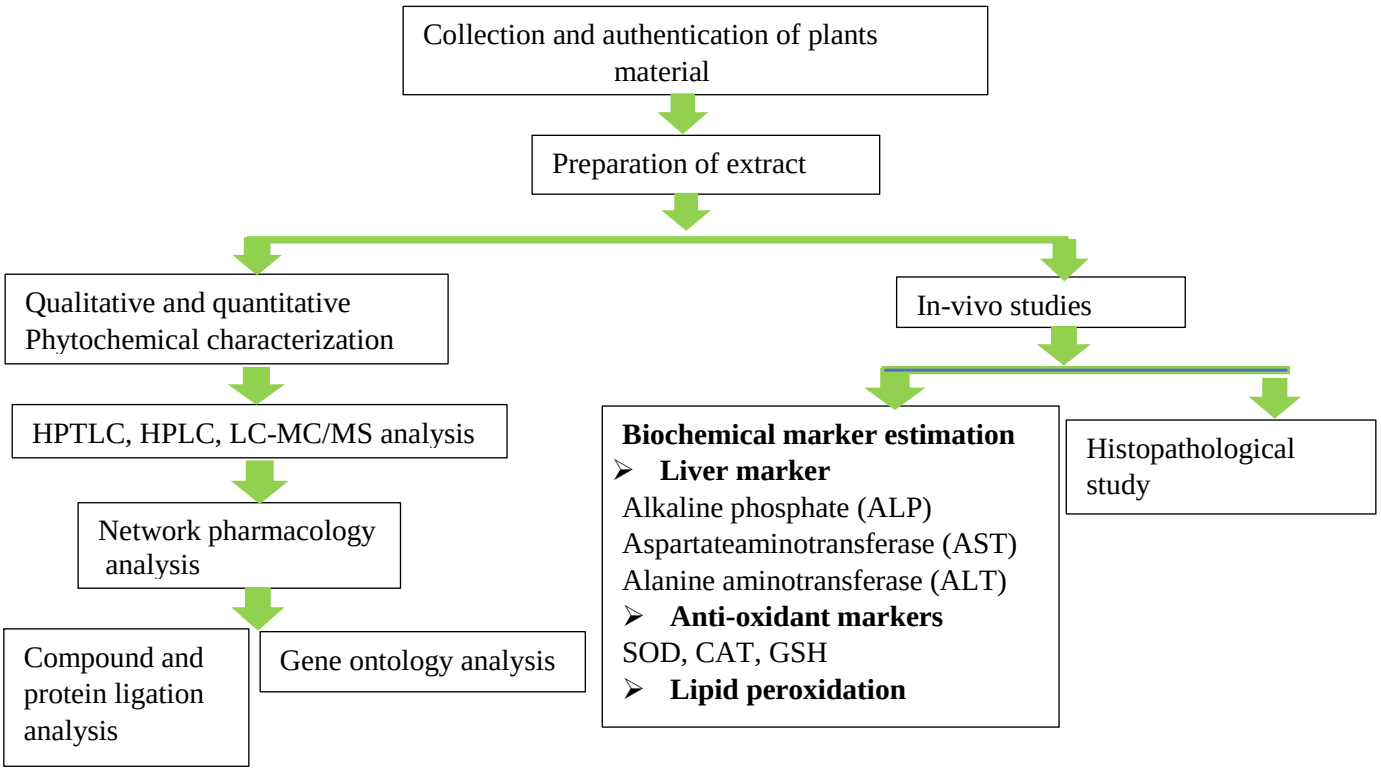
4. AIM

Development of quality standard and hepatoprotective evaluation of Indian medicinal plants.

OBJECTIVES

- Collection and drying, authentication and extraction of selected plant material.
- Quality guidelines for medicinal plants are being developed using cutting-edge analytical methods like UV, HPTLC, HPLC/LC-MS, etc.
- In vivo evaluation of hepatoprotective activity against carbon tetra chloride in rat model.
- Investigation of medicinal plants' multi-targeted therapeutic benefits for the treatment of liver disease.

5. PLAN OF WORK



6. COLLECTION AND AUTHENTICATION OF PLANT

Plant material will be collected from the neighbour area or market and will be authenticated.

Pharmacological evaluation: Pharmacological evaluation can be done for detailed studied.

(a) Macroscopic study: The macroscopic evaluation of drug classified by their organoleptic character. Systematic examination of the colour, odour, taste, size, shape etc both the plant was carried out.

(b) Microscopic assessment: The microscopic assessment of medication types under a microscope Systematic inspection of plant products like starch granules, fibers, calcium oxalate crystals, stone cells, etc. was done.

Initial phytochemical testing

Both plants' freshly prepared extracts have been put through a phytochemical screening test to look for different constituents. Alkaloids, glycosides, flavonoids, tannin, saponin, and other compounds were found.

Physical evaluation of plants: Their standard technique was employed to figure out the quantity of foreign organic matter, moisture content, total ash, water soluble ash, acid insoluble ash, extractive value, etc.

Phytochemical investigation: Their standard procedure involved completing a phytochemical constituent on both plant extracts using cutting-edge analytical tools like a UV spectrophotometer, HPTLC, and LC-MS/GC-MS.

Network pharmacology analysis

Network models

The basis for analysing interaction in sophisticated networks is network modelling. Network structures produced by different graph-based models are employed to compare for actual elaborate networks. Network models like the cascade model, niche model, as well as ecopath model have been designed for food webs. The system variables, such as biomasses, predation, and output, can be dynamically depicted. Some model parameters are also network models, and they can be adjusted and improved to accommodate pharmacological networks.

(WenJun Zhang., 2016).

Gene Ontology (GO) analysis

Multiple pathophysiological genes in DN were assessed to use a gene ontology (GO) study. The analysis was carried out with the aid of Metascape and a network analyst tool. It implicated the genes that were discovered to communicate with active metabolites. By reducing endothelial dysfunction, fatty liver disease, diabetes mellitus, acute kidney injury, fibrosis,

hypertension disease, obesity, etc., it illustrates the numerous physiological functions of each objective in the modulation of DN. Additionally, it was revealed that the goal potential is critical for controlling oxidative stress, inflammation, and inflammatory processes driven on by oxidative stress. **(Q. Tao et al., 2020, L Cheng et al., 2021, Q. D. Xia et al., 2020).**

Protein ligation

Dithiothreitol, mercaptoacetic acid, N-acetylcysteine, and cysteine are employed as cofactors at a concentration of 100 mm and 1.55 v/v (thiophenol). actually, occurred Dithiothreitol, thiophenol, as well as cysteine in 200 mm of NaCl and 0.5 M of phosphate solution, pH 7.3 (N-acetylcysteine and mer-capto acetic acid). Gently stirring all of the reactions overnight before diluting them ten times with transcription buffer (20 mm tris-HCL, pH 7.9, 100 mm KCL, 10 mm MgCL2). To reduce proteolytic degradation, all of the buffers were added with 1 mm phenyl methyl sulfonyl fluor and then diluted. The experiment is conducted in a cold environment. The supernatant was dialyzed against two 1-liter changes of transcription buffer after the beads were permitted to settle. On a C-30 concentrator, the protein has been concentrated. **(Konstantin S et al., 1998).**

7. BIOLOGICAL SCREENING

Hepatotoxicity Activity

Both plant extracts were evaluated for prospective hepatoprotective impacts using carbon tetra chloride, and silymarin served as the comparative medication.

Reagent Composition:

Carbon tetra chloride (CCl4) and Silymarin

Standard Assay Set

Alkaline phosphatase, Alanine transaminase, and Aspartate transaminase (ALP), LDL overall, Triglycerides, Glucose, Bilirubin, serum creatinine, bilirubin, and high-density lipoprotein (HDL)

Animals: Hepatoprotective activity was evaluated on Wistar albino male rats (150-250 gm). All the animals were kept in the group of 6 in a clean polypropylene cage at 23±ºC and acclimatized for 7 days under day and night cycle. Standard feed and water were given to all the animal at proper timings before start the experimental work.

Oral Acute Toxicity Study: Animal were divided into three groups (three each) and administrated with extracts to Group I (1000 mg/kg), group II (2000 mg/kg), group III (4000 mg/Kg) with oropharyngeal cannula. All the animals were free to take proper food and water intake, however all the animals were deprived from the food for 2.5 hrs before or after 4 hrs dose. Immediately after given dose each animal was observed continuously for 15 minutes interval in first 4 hrs for physiological, psychological and finally mortality end of 24 hrs and 14 days.

Groups	Number of animals	Treatment	Dose	Route	Duration
A I	3	Extract and standard drug	1000 mg/kg body weight	Oral	14 days
II	3		2000 mg/kg body weight		14 days
III	3		4000 mg/kg body weight		14 days
BI	3		1000 mg/kg body weight		14 days
II	3		2000 mg/kg body weight		14 days
III	3		4000 mg/kg body weight		14 days

Carbon tetra chloride (CCl4) induced hepatotoxicity:

All the animals were kept on the same number of calories so all the calories should not be factor to alter the effect of CCl4 induced hepatotoxicity. First day AST and ALT were measured before given oral dose of CCl4 and again measured after 07 days of intra peritoneal dose of CCL4. Increased AST

and ALT level compared to first day was considered to be a liver toxicity in rats which is used in the experiment **(Shenoy K. A et al., 2001)**

S No.	Group	Treatment	Dose
1.	Group I	Control	Received water (5ml/kg. p.o) for 21 days once daily, and served as normal control
2.	Group II	Negative control	Received water (5ml/kg. p.o) and 40% aqueous water v/v (2.0 ml/100 g body wt., p.o.) for 21 days
3	Group III	Standard	Received 40% aqueous water v/v (2.0 ml/100 g body wt., p.o) for 21 days and standard drug silymarin (25 mg/kg. p.o) for 21 days once daily
4.	Group IV	High dose	Received 40% aqueous water v/v (2.0 ml/100g body wt., p.o) for 21 days
5.	Group V	Low dose	Received 40% aqueous water v/v (2.0 ml/100g body wt., p.o) for 21 days

After the completion of 14 days of experiment blood sample of rats were collected from the tail vein. Collected blood sample were centrifuge at 4000 rpm for 10 min, separate the fresh serum in clean, tight closed container and stored in refrigerator. Antioxidant markers such as SOD, CAT, GSH and lipid peroxidation and biochemical parameters determined by using commercial Kit **(Adamu A. et al., 2009).**

Procedure of Liver marker estimation

- **Alkaline Phosphate (ALP)**

  Add 0.05 ml of the sample to 3 ml of the reagent in the micro dish using a pipette. It mixes correctly, and after reading the starting absorbance that was logged, the stopwatch is initiated. All samples were stored at 4°C and tested in under 72 hours of collection. It was measured even after 1, 2, and 3 minutes.

  Aspartate aminotransferase (AST)

  Aspartate amino transferase analyzes the reaction product of pyruvate and 2,4-dinitrophenyl hydrazine at 546 nm. The rate of NADH consumption with respect to ALT enzyme activity is evaluated at 340 m. Take 100 L of the serum and blend it before 30 minutes of incubation at 37 °C. The reacting vessel acquired 500 L of developer (2,4-dinitrophenylhydrazine), was stirred, and then left at room temperature for 20 minutes. The tube was then filled with 5 ml of 0.4 N NaOH and kept at room temperature for five minutes. In a light path cuvette, the test materials' spectrophotometric absorbance was determined against a water blank at a distance of five hundred m. assessing the test materials' absorbance in reference to a manual calibration number.

Alanine transferase (ALT)

When pyruvate reacts with 2,4-dinitrophenyl hydrazine, pyruvate forms pyruvate hydrazine, which is detected by alanine transferase at 546 nm. The rate of NADH use with regard to ALT enzyme activity is assessed at 340 m. Take 100 L of the serum and blend it before 30 minutes of incubation at 37 °C. The reacting container acquired 500 L of developer (2,4-dinitrophenylhydrazine), was stirred, and then left at room temperature for 20 minutes. The tube was filled with 5 ml of 0.4 N NaOH and kept at room temperature for 5 min. In a light path cuvette, the test materials' spectrophotometric absorbance was measured against with a water blank at a distance of 500 m. comparing the test samples' absorbance to a calibration number listed in the manual of the instrument

Antioxidant makers

- **Estimation of superoxide dismutase (SOD)**

  EDTA 0.1 nm, sodium carbonate 50 nm, and neon blue tetrazolium are good examples (NBT). 0.05 ml of hydroxylamine and 0.05 ml of PMS were added to 2 ml of the combination in a cuvette, and the auto-oxidation at 560 nm, hydroxylamine gauges absorbance.

- **Estimation of catalase (CAT)**

  In a final volume of 3.0 ml, blend 1.95 ml of phosphate buffer (0.05 M, pH 7), 1.0 millilitre of hydrogen peroxide (0.019 M), and 0.05 ml of PMS (10%). If there are any changes in absorbance, record the measurement at 240 nm. The catalase activity was determined in K minutes-1.

- **Estimation of reduced glutathione (GSH)**

  1.0 ml of PSM (10%) and 1.0 millilitre of sulphosalicylic acid (4%), respectively, were precipitated. The samples were centrifuged at 1200 grams for fifteen minutes at 4^0C after being stored at 4^0C for at least an hour. 3.0 ml of phosphate solution (0.1 M, pH 7.4) and 0.1 ml of filtered aliquot composed the assay combination. On a spectrophotometer, the yellow-colored produced was read straight away at 412 nm.

- **Estimation of Lipid peroxidation**

  Take 50 milligrams of tissue samples, homogenized them in 0.1 M phosphate buffer at room temperature (pH 7), and centrifuge the mixture for 15 minutes at 3000 rpm. The supernatant (100 l) and 20 millimeters guaiacol were combined. The time period was recorded for the 0.1 absorbance rise at 436 nm in the presence of 300 l H2SO4 (12.3 mm).

HISTOPATHOLOGICAL INVESTIGATION

Procedure for histopathology of liver

For the histological evaluation, tiny pieces of liver tissue have been recovered in 10% formaldehyde solution. The pieces of liver that had been processed for 48 hours in 10% formalin saline were successfully dehydrated by moving through three distinct ethanol-water mixtures before ending up in absolute alcohol (50, 80, and 95%). The liver tissue was cut into sections that were 4-6 mm thick and embedded in paraffin wax. For microscopic analysis of cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, and infiltration of Kupffer cells and lymphocytes, they were stained with hematoxylin and eosin. **(Drury et al., 1976).**

EXPECTED OUTCOME

- Liver disease and its associated pathophysiology exponentially concerning to the healthcare system.
- Development of the most effective and therapeutically active phytopharmaceutical is needed for treatment of liver disease.
- In this study, a phytopharmaceutical will be developed to alleviate liver disease and associated pathophysiological conditions.
- The developed phytopharmaceutical will be most accessible, affordable and economic in nature that would promote sustainable development of the Nation.

TIME LINE

Objectives	1-6	6-12	12-18	18-24	24-30	30-36
Collection and authentication of medicinal plants						
Preparation of alcoholic extracts						
Incorporating cutting-edge analytical methods like a UV spectrophotometer, HPTLC, and LC-MS/GC-MS, qualitative and quantitative phytochemical assessment of prepared extracts has been accomplished.						
In-vivo hepatoprotective analysis of prepared extracts against CCl4 induced hepatotoxicity on Wistar rats.						
Research of the multi-targeted therapeutic role of medicinal plants to use in auto dock analysis and writing, network pharmacology, and writing.						

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